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SUSCEPTIBILITY OF HIBERNATING CODLING MOTH LARVAE TO LOW TEMPERATURES, AND THE BOUND-WATER CONTENT [1]

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INTRODUCTION

The codling moth (*Carpocapsa pomonella* (L.)) is a pest of apples wherever they are grown in the United States. It is able to survive under a diversity of climatic conditions, prominent among which are low winter temperatures. At times, however, overwintering larvae are killed by exposure to low temperatures. At Yakima, Wash., for example, Newcomer (*5*) [3] found that wherever the minimum temperature had been lower than −25° F. all overwintering larvae with no protection other than bark or burlap bands were killed.

A study was therefore undertaken to find out what low temperatures are fatal to larvae of the codling moth, whether larvae indigenous to widely separated apple-growing districts show any differences in susceptibility to cold, and whether cold-hardiness is influenced by differences in sex or weight of the larvae or by differences in humidity of the atmosphere before exposure to cold. Determinations were also made of the bound-water content of hibernating codling moth larvae, since bound water is generally believed to have a part in cold-hardiness.[4]

THE FREEZING OF INSECTS

Some insects can be cooled considerably below their true freezing temperature before actually freezing. The point reached just as freezing commences is referred to as the undercooling temperature. When the first ice crystal is formed, the heat of crystallization causes a sudden rise, the limit of which is called the rebound temperature. The temperature of the insect then remains constant for a moment, until the body fluids solidify, and then falls again to the temperature

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[3] Italic numbers in parentheses refer to Literature Cited, p.340.

[4] Because of space limitations many details of procedure, the formulas used for computations, and certain data have been omitted. Any desired information on these details will be furnished by the author on request.

of the surrounding media. The true freezing temperature of the insect is somewhat higher than the rebound temperature, because part of the heat of crystallization is transferred to the surrounding media. No attempt has been made in this study to determine the true freezing temperature of the codling moth. A typical cooling curve for the codling moth is shown in figure 1.

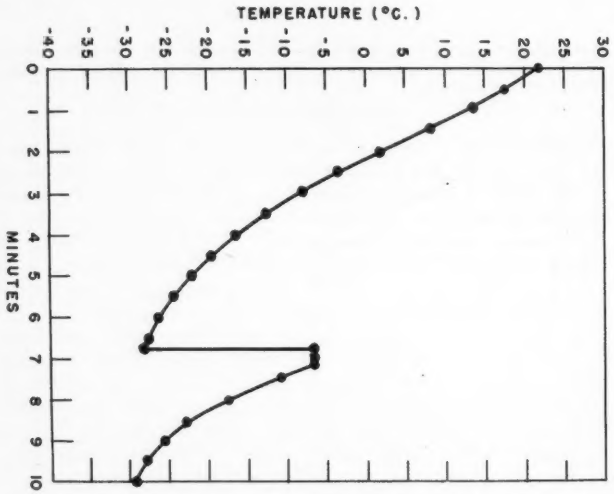

FIGURE 1.—Typical cooling curve for the codling moth larva, showing undercooling and rebound temperatures.

Several theories have been advanced to explain the part freezing plays in killing insects. An early view that the formation of ice in the cells causes death through mechanical injury has largely been abandoned. Some investigators believe that withdrawal of water from the cells is responsible, others that the normal functionings of the cell walls are destroyed.

Bachmetjew (*1*), one of the first entomologists to study the cold-hardiness of insects, reported that death occurred only after the insect had been subjected to its undercooling temperature a second time. As found by the author, and as pointed out by Salt (*11*) and others, some insects can survive freezing, and are killed only by temperatures below their undercooling point.

Robinson (*8*, *9*) studied the possible correlation between the hydrophilic colloid content of an insect and its winter-hardiness. Some investigators believe that such colloids adsorb sufficient water to lower the fatal temperature. This adsorbed water is called bound water, whereas the other water in the insect is known as free water. The properties of water associated with colloids are known to differ from those of true solutions. Thoenes (*13*) stated that bound water will not freeze at −20° C., and Jones and Gortner (*3*, *p. 434*), using the dilatometric method, found that the bound water present in

gelatin and the white of egg did not freeze even at −50° C. These authors state:

> It is emphasized that "bound" water is an indeterminate term, and that "bound" water values as experimentally determined may be expected to vary from system to system, the variation being due to many factors, not the least of which is the method selected for measurement. If biological cells and tissues are similar in their behavior to gelatin and (probably) to the thick portion of egg white, then "bound" water is a measurable entity and (using dilatometric procedure) has a constant value at least at temperatures between −6° and −50° C.

On the basis of these theories Robinson (*8*, *9*, *10*) made studies of the proportion of bound water present in several species of insects. Kistler (*4*), however, has questioned the validity of results based on these hypotheses, on the ground that the undercooling of some of the water would interfere with their accuracy. More recently Ditman, Vogt, and Smith (*2*, *p. 272*) have expressed the opinion "that unfreezable water determinations are of no value in estimating the relative cold-hardiness of insects."

STUDIES OF UNDERCOOLING AND REBOUND TEMPERATURES

COLLECTION OF LARVAE

Representative overwintering larvae from different sections of the United States were obtained by banding apple trees in fruit districts in five States, as follows: Maryland (Easton), Georgia (Cornelia), Arkansas (Bentonville), Colorado (Grand Junction), and Washington (Yakima). The bands containing the larvae were shipped to Takoma Park, Md., where they were held in an outdoor insectary until used in these studies.

APPARATUS AND PROCEDURE

The apparatus for determining the undercooling and rebound temperatures consisted essentially of a refrigerator for cooling the larvae and a temperature-recording device (fig. 2).

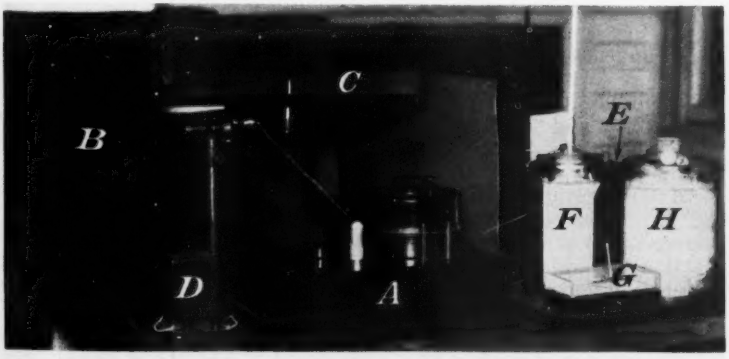

FIGURE 2.—Apparatus used in determining undercooling and rebound temperatures of codling moth larvae: *A*, Potentiometer; *B*, hood for galvanometer (not visible) and scale; *C*, scale for galvanometer reading; *D*, standard cell; *E*, battery; *F*, cold junction; *G*, mercury switch; *H*, vacuum-bottle refrigerator.

The refrigerator (*H*) was a wide-mouth vacuum bottle supported within a cotton-filled battery jar. A cell (fig. 3) for holding the insect was made out of two corks, one larger than the other. The smaller end of one cork was hollowed out enough to hold the insect and inserted in a pocket in the larger cork which had been cut out and filled with cotton. Thermocouple wires were inserted through the smaller cork so that the junction would be in contact with the larva. The cell was then placed in a container consisting of two test tubes one inside the

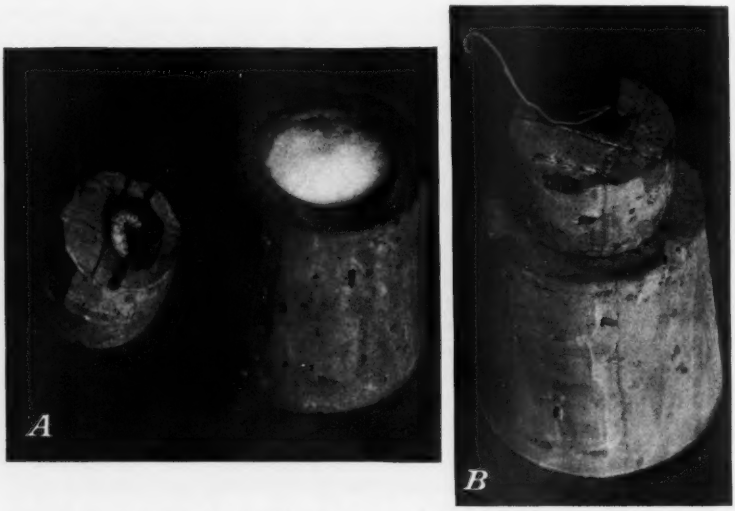

FIGURE 3.—Insect cell for determination of undercooling and rebound temperatures of the codling moth larva: *A*, Cell open showing position of larva and thermocouple wires; *B*, cell closed.

other, and inserted in the vacuum bottle through a hole in its cork stopper.

To produce low temperatures a eutectic mixture of calcium chloride hexahydrate and finely crushed ice or a mixture of solid carbon dioxide and gasoline was used. The lowest temperature obtained with the first mixture was −37° C., although it is capable of giving −54.9°.

All temperature measurements were made with a potentiometer (fig. 2, *A*) in connection with a D'Arsonval galvanometer and a thermocouple of copper-constantan wire. The cold-junction ends of the thermocouple were lowered into small glass tubes containing a half-inch of mercury, which were inserted through a cork stopper into a pint vacuum jar filled with finely crushed ice (*F*). To reduce electrical resistance a home-made mercury-pocket reversing switch (*G*) was employed in place of a knife switch. The thermocouple was calibrated as to electromotive force-temperature function in accordance with a standard equation and constants.

EXPERIMENTAL RESULTS

In the tests reported in this paper no codling moth larvae survived after one exposure to their undercooling temperature, although a few individuals lived in a very weak condition for several weeks following their freezing.

Effect of Locality, Sex, and Weight

The undercooling and rebound temperatures of larvae from several fruit districts in the United States are summarized in table 1. Practically no differences due to locality of origin are indicated by these data.

Table 1.—*Relation of locality of origin of codling moth larvae to their undercooling and rebound temperatures, 1931*

State	Larvae	Mean undercooling temperature	Mean rebound temperature
	Number	*° C.*	*° C.*
Maryland	3	−26.0±1.4	−6.0±0.4
Colorado	11	−25.9± .7	−6.2± .5
Arkansas	5	−27.0± .6	−7.4± .3
Georgia	21	−25.8± .6	−6.7± .6
Washington	23	−26.0± .6	−6.9± .8

Similar data for other groups of larvae separated according to sex are given in tables 2 and 3.

Table 2.—*Relation of locality of origin, sex, and weight of codling moth larvae to their undercooling temperatures, 1932*

State	Sex of larvae	Number of larvae	Mean weight	Mean undercooling temperature
			Gram	*° C.*
Georgia	Male	6	0.039	−28.3±0.6
	Female	9	.047	−25.5±1.6
Maryland	Male	5	.039	−26.4±1.8
	Female	10	.050	−25.6± .6
Washington	Male	9	.039	−26.1± .5
	Female	11	.052	−27.2± .3

Table 3.—*Relation of locality of origin, sex, and weight of codling moth larvae to their undercooling and rebound temperatures, 1933*

State	Sex of larvae	Number of larvae	Mean weight	Mean undercooling temperature	Mean rebound temperature
			Gram	*° C.*	*° C.*
Maryland	Male	3	0.054	−27.7±0.9	−8.8±1.1
	Female	1	.059	−28.3	−5.0
Colorado	Male	6	.035	−29.4± .7	−11.1±1.3
	Female	5	.041	−29.3± .5	−11.2±1.2
Arkansas	Male	2	.041	−28.4±1.7	−8.0± .8
	Female	2	.046	−28.9±1.0	−9.5± .8
Georgia	Male	4	.047	−30.1± .7	−9.3±2.0
	Female	7	.055	−27.3±1.0	−6.5± .7
Washington	Male	2	.041	−24.4±1.6	−7.2±2.1
	Female	2	.042	−26.5±3.0	−9.7±1.7

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The results presented indicate that neither the undercooling nor the rebound temperature is appreciably influenced by the locality from which the insects are obtained or by the weight or sex of the larvae. These conclusions are supported by other work, presented in the next section, although the data for individual localities are not given.

Effect of Dry and Humid Conditions

Payne (*6*), in experiments with *Synchroa* larvae, demonstrated that both the undercooling and the freezing temperature could be lowered by dehydrating the larvae. This was true whether or not the insects were normally self-dehydrating. Payne also found (*7*) that the larva of the Japanese beetle (*Popillia japonica* Newm.), which normally overwinters in a moist environment, is very resistant to cold when dehydrated to half its body weight.

A series of tests was conducted in which larvae, undisturbed in their cocoons, were held at 10° C. in sealed glass containers for 1 to 4 months, some under humid and others under dry conditions, before their undercooling and rebound temperatures were taken. Moisture was supplied by means of lampwicks dipped into a beaker of water. For tests under dry conditions the air in the container was dehydrated with silica gel, which was thoroughly dried out weekly. The exact extent of the dehydration was not determined for these larvae. Because of the codling moth larva's habit of spinning new cocoons if disturbed, it was not possible to weigh naked larvae before and after dehydration without affecting the normal condition of the insect.

The summarized results, given in table 4, indicate that the average undercooling and rebound temperatures of the insects were not markedly affected by the extremes in humidity under which the larvae had been kept. It is believed, therefore, that the resistance of the dormant codling moth larva to low temperatures is not greatly changed by wide differences in humidity such as sometimes exist under field conditions.

Table 4.—*Undercooling and rebound temperatures of hibernating codling moth larvae that had been subjected to dry and humid conditions previous to exposure to low temperatures*

Atmospheric condition previous to test	Sex of larvae	Number of larvae	Average weight	Mean undercooling temperature	Mean rebound temperature
			Gram	° *C.*	° *C.*
Dry	Male	9	0.036	−25.6	−8.3
	Female	16	.051	−26.8	−7.5
Humid	Male	13	.044	−25.7	−7.1
	Female	12	.053	−27.7	−7.9

Effect of Cross-Breeding

To determine the effect of cross-breeding on resistance to low temperatures, male moths from Washington were crossed with females from Georgia. As far as could be determined from five crossbred males, and six crossbred females, crossing produced no change in the susceptibility of the resulting larvae to low temperatures.

PROTECTIVE VALUE OF UNDERCOOLING

As the temperature falls, the larva lies quiet in its cocoon, and its metabolic processes become for the most part inactivated. This state of rest perhaps favors undercooling in the same way that quiet conditions permit water to be undercooled considerably below 0° C. There is no chance for foreign substances to be introduced into the body fluids of the larva to serve as nuclei for ice formation. Moreover, the host tree, on which the larva is quartered beneath the bark, is relatively free from vibrations.

Since the temperature required to produce the first crystal of ice is much lower than the true freezing temperature, the ability to undercool permits the insect to withstand winter temperatures many degrees below its true freezing temperature.

To determine whether the inactivity of the larvae during the winter facilitates undercooling, tests were conducted with three larvae from Maryland and four from Arkansas. As the temperature was lowered, the larvae were kept in motion by means of a wire looped around their thoracic segments, while the thermocouple remained in contact with the abdominal segments. Both the undercooling and the rebound temperatures were considerably higher than when the larvae were undisturbed. The fact that undercooling occurred at all is evidence that the tendency to undercool is exceedingly pronounced.

DETERMINATIONS OF BOUND WATER

Bound-water determinations were made by two methods. The first was the dilatometric method, which had been used by Jones and Gortner (*3*) for various gels and by Sayre (*12*) for plant tissue. The second method was the heat-of-fusion-of-ice method, which had been used by Robinson (*10*) and others.

DILATOMETRIC METHOD

The dilatometric method takes advantage of the expansion that occurs with the freezing of water to measure the quantity of water that freezes in the larvae. The total water content is then determined by drying the larvae, and the bound water is determined by difference.

The dilatometer used (figs. 4 and 5) consisted of a graduated capillary tube of 1-mm. bore and 55 cm. long fused to a 25-ml. bulb into which was ground a glass stopper provided with ears for the attachment of rubber bands. Toluene was selected as a suitable liquid, since it is practically insoluble in water and has a low freezing point. Two glass containers, one inside the other, separated by 3 inches of ground cork formed an insulated chamber, which was filled with calcium chloride solution. This chamber was equipped with a motor-driven stirrer.

Approximately 125 codling moth larvae, having a total weight of about 5 gm., were used in each test. After being weighed they were introduced into the dilatometer bulb containing toluene. Additional toluene was then drawn in through the capillary tube with an aspirator to a certain scale reading, while the glass stopper, smeared

with glycerol, was slowly revolved until it seated, care being taken to exclude air bubbles. As extra precautions rubber bands were used and the stopper was sealed with celluloid cement.

The calcium chloride bath was cooled to and kept constant at −7.5° C. This temperature was selected because it is between the maximum undercooling temperature and the freezing temperature of the larvae.

The dilatometer containing the larvae was then placed in the bath, and when thermal equilibrium was established the reading was taken. The dilatometer was next transferred to a freezing chamber at −35° C. and held therein until all the larvae were frozen, when it was replaced in the bath at −7.5° C. The quantity of water that was frozen within the larvae was computed from the difference in the readings of the dilatometer when the larvae were in the unfrozen and the frozen state. The larvae were then removed from the dilatometer and placed in an electric oven at 87°–89° C., where they were desiccated under vacuum until a constant weight was obtained. The total quantity of water originally present in the larvae was then calculated. From

FIGURE 4.—*A*, Dilatometer; *B*, insulated temperature chamber; *C*, stirrer; *D*, rheostat; *E*, thermocouple.

these two figures was computed the percentage of the total water that was in the bound form.

The results with five tests at −7.5° C. showed the bound-water content to range from 34.4 to 38.4 percent, with a mean of 35.9 percent.

HEAT-OF-FUSION-OF-ICE METHOD

The heat-of-fusion-of-ice method was essentially that reported by Robinson (*10*). This method utilizes the fact that the melting of

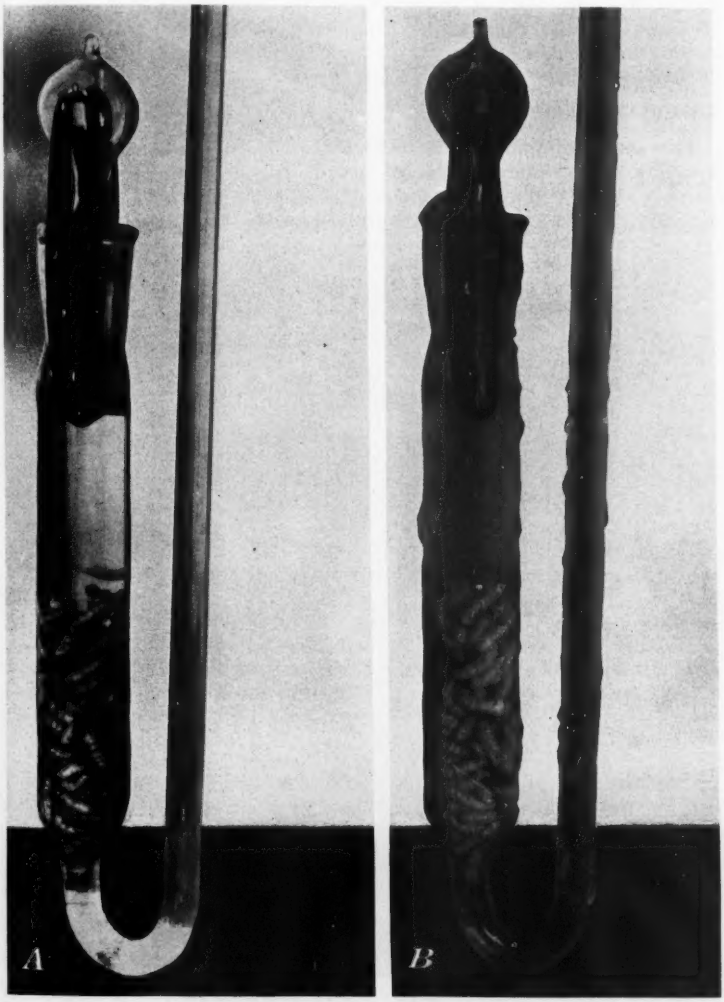

FIGURE 5.—Dilatometer bulb showing codling moth larvae (*A*) before freezing, and (*B*) after freezing. Note whitish color of larvae caused by freezing.

1 gm. of ice requires 80 calories of heat. By the use of a calorimeter (fig. 6) the heat given off by the thawing of frozen larvae is measured. This figure gives a basis for computing the frozen water that they contained. The total water content is then determined as was done in the dilatometric method, and the bound water computed by difference.

A 300-ml. silvered Dewar flask (*A*) was used as the calorimeter jacket. A small glass shell vial (*E*), held in place within the flask by means of a cork, served as a container for the calorimeter water. In all tests the water was carefully pipetted into the vial to avoid drops on the walls. A mechanical stirrer (*B*) was made from a glass rod by flattening it at one end and twisting it spirally to give adequate stirring without splashing. It was operated by a $\frac{2}{200}$-horsepower motor.

The water sample used to determine the thermal capacity of the calorimeter and the samples of insects to determine the free-water content and specific heat were enclosed in tinfoil containers (fig. 7). A thermocouple was held in the middle of the insect samples.

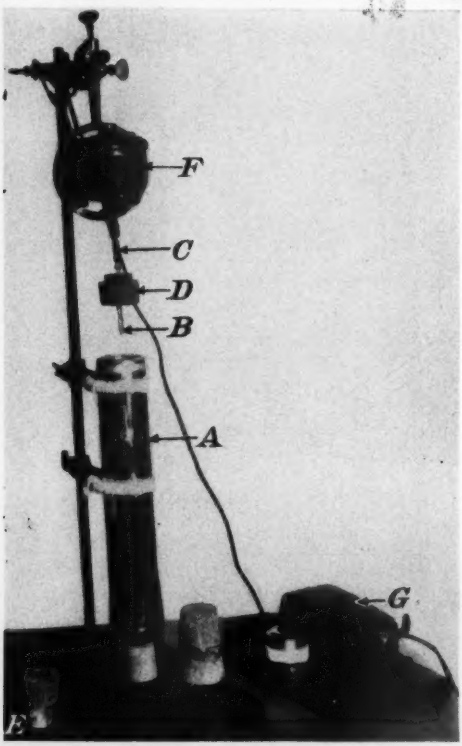

FIGURE 6.—Calorimeter, open: *A*, Dewar flask; *B*, glass stirrer; *C*, universal joint; *D*, cork stopper for Dewar flask and bearing for stirrer; *E*, container for calorimeter water; *F*, motor for stirrer; *G*, rheostat for motor.

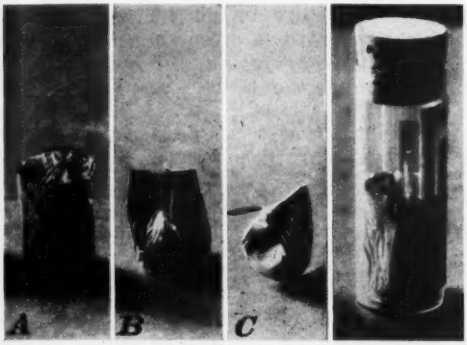

FIGURE 7.—Tinfoil container for specimens used in bound-water determinations by the heat-of-fusion method: *A*, tinfoil cylinder; *B*, cylinder after folding for freezing of specimens; *C*, cylinder as folded after specimen was frozen; *D*, glass vial container for specimens.

TABLE 5.—*Bound-water content of codling moth larvae, determined by the heat-of-fusion-of-ice method*

Temperature of frozen larvae, −30° C. Specific heat of larvae, 0.75 calorie per gram-degree centigrade

Weight of live larvae	Water content of larvae	Weight of tinfoil container	Temperature of calorimeter water		Bound water
			Initial	Final	
Grams	*Percent*	*Grams*	*°C.*	*°C.*	*Percent*
2.530	54.9	1.636	26.0	20.1	11.2
2.508	54.6	1.360	22.7	17.0	8.4
2.512	56.1	1.266	25.2	19.3	14.8
2.521	56.5	1.238	25.6	19.5	5.3
2.529	56.2	1.266	24.9	19.0	10.5
2.518	56.8	1.278	24.7	19.0	16.3
2.509	56.7	1.207	24.6	18.7	9.5
2.504	55.8	1.220	24.6	18.9	11.9
2.505	55.1	1.269	24.8	19.1	12.5
2.504	56.3	1.250	24.8	19.0	11.2
Mean 2.514	55.9	1.299	24.8	19.0	11.2

The results of 10 bound-water determinations by this method are given in table 5.

It will be noted that a lower value for bound water was obtained with the heat-of-fusion method at −30° C. than with the dilatometric method at −7.5°. A difference in results should exist, since the force of crystallization is more powerful in comparison with the force of water binding at −30° than at −7.5°.

SUMMARY

Studies have been made of the susceptibility of the hibernating larva of the codling moth (*Carpocapsa pomonella* (L.)) to low temperatures, as indicated by the temperature to which the insect can be undercooled before beginning to freeze. Studies have also been made, by two methods, of the bound-water content of the hibernating larva.

Unlike some insects, the codling moth cannot withstand more than one freezing.

The overwintering larva shows a pronounced tendency to undercool. The average undercooling temperatures for different groups of larvae ranged from −24.4° C. to −30.1°. The average rebound temperatures to which the temperatures of the insects rose after freezing started ranged from −5.0° C. to −11.2°.

No marked differences in susceptibility to low temperatures, as indicated by the undercooling temperatures, were shown by larvae from Washington, Colorado, Arkansas, Maryland, and Georgia.

The undercooling temperatures of the larvae were practically unaffected by the sex or weight of the larvae.

A study of the effect of exposing larvae to artificially created dry and humid conditions indicated no marked changes in their undercooling temperatures.

The undercooling temperatures of crossbred larvae were similar to those of their progenitors.

Undercooling is favored by the quiescent state of the larva during its hibernation period. The artificial movement of the larva greatly reduced undercooling.

The average bound-water content of codling moth larvae was 35.9 percent when determined by the dilatometric method at −7.5° C., and 11.2 percent by the heat-of-fusion-of-ice method at −30° C.

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